IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Rolf J. Mehlhorn	Group Art Unit: 1615
Application No.: 08/472,843	Examiner: Gollamudi S. Kishore
Filed: June 7, 1995	Appeal No.:
For: METHOD FOR LOADING LIPID LIKE VESICLES WITH DRUGS OR OTHER CHEMICALS	

BRIEF FOR APPELLANT

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This appeal is from the decision of the Primary Examiner dated December 20, 2002 (Paper No. 34), finally rejecting Claims 46-65, which are reproduced as an Appendix to this brief.

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The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.

I. Real Party in Interest

The present application is assigned to The Regents of the University of California.

II. Related Appeals and Interferences

The present application is a divisional application of U.S. Application Serial No. 07/741,305, filed August 7, 1991 ("the parent application"). Interference 103,469 was declared on October 11, 1994 between the parent application and U.S. Application Serial No. 07/393,118, filed August 4, 1989 ("the Forssen application"). However, the claims involved in the interference differed from those which are currently pending. Likewise, the combination of prior art being argued in the interference likewise differs from that currently being applied. Finally, the Decision in Interference 103,469 was based on the Request for Adverse Judgment filed by Applicants in accordance with a settlement reached with the opposing party. No final Judgment by a three-member panel of the Board was ever entered.

III. Status of Claims

Claims 1-45 are canceled. Claims 46-65 are pending. Claims 46 and 52 have been twice amended.

IV. Status of Amendments

The claims stand as they were amended on October 10, 2002. Applicant further amended the claims on January 20, 2004, but the amendments were not entered by Examiner Kishore.

V. Summary of the Invention

The present invention is based on the surprising discovery that the use of pH gradients, in accordance with the invention, to load liposomes allows for the rapid uptake of drugs or chemical species by the liposomes. In addition, the chemical-species loaded liposomes of the invention are stable in the presence of the pre-imposed pH gradient and in the absence of the pre-imposed pH gradient. The claims require that the stability of the liposomes is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species." This unexpected feature allows the liposomes to remain stably loaded after they are injected into a host where the pre-imposed pH gradient no longer exists. This feature of the present invention is important for preventing massive amounts of drug from leaking or being "dumped" in the host after administration of the drug loaded liposomes. Moreover, it allows for the safe administration to animals, because the lack of leakage causes the animal to suffer "no long-term effects of the administration" (as recited by Claims 46 and 52).

Applicant has experimentally shown that it is not necessary to maintain the pH gradient to keep the liposomes in a loaded state. Example 3 of the present

application demonstrates that when the liposomes were placed in a ten-fold excess of lysine buffer, the pH gradient that had been preimposed for loading was largely collapsed, and as a result, very little leakage of the entrapped chemical occurred. Moreover, Example 2 of the present application demonstrates that animals suffer no long-term effects of the liposome infusion.

VI. The Issues

1. Whether Claims 46-54, 56-57 and 61-64 are anticipated under 35 U.S.C. §102(b) as being anticipated by Nichols.

2. Whether Claims 46-54, 57 and 61-64 35 U.S.C. § 102(b) are anticipated by Deamer et al.

3. Whether Claims 46-54, 59 and 61-64 are anticipated under 35 U.S.C. § 102(b) by Cramer et al. or Kano et al.

4. Whether Claims 46-64 are unpatentable under 35 U.S.C. § 103(a) over Nichols et al., Deamer et al., Cramer et al. or Kano et al.

VII. Grouping of Claims

The claims do not stand or fall together. In particular, Claims 46 and 52 are directed to methods for preparing a stable liposome vesicle-entrapped chemical species, and should be grouped with Claims 48 and 61, wherein the aqueous medium is a buffer solution, and Claims 49 and 62, to liposome vesicle-entrapped

chemical species prepared by the method of Claim 46. As such, Claims 46, 48, 49, 52, 61 and 62 can be grouped together.

However, Claims 47 and 53 should be considered separately from Claims 46, 48, 49, 52, 61 and 62, because Claims 47 and 53 are directed to the methods of Claim 46 or 52, respectively, wherein the chemical species is a drug. Likewise, Claims 50 and 63 are directed to pharmaceutical preparations for administration *in vivo* to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49 or 62, respectively. Furthermore, Claim 54 is directed to a method wherein the liposomes comprise a hydrophobic drug, and Claims 57 and 59 are directed to methods wherein the drugs are basic, or acidic, respectively. None of the cited references disclose or suggest the preparation of liposomes containing drugs for delivery to animals. As such, Claims 47, 50, 53, 54, 57, 59 and 63 can be grouped together.

Claims 51 and 64 can also be grouped separately, because they are directed to pharmaceutical preparations for parenteral administration *in vivo* to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49 or Claim 62, respectively, wherein the buffer has an osmolarity within the physiological range of an animal, the vesicles are suspended for administration in a bulk solution, and the bulk solution has a pH which is physiologically benign. As such, these preparations must be made using a step of suspending the liposomes in that physiologically benign pH buffer. None of the cited references disclose suspending the loaded liposomes in a physiologically benign pH buffer. As such, Claims 51 and 64 can be grouped together.

Finally, the remaining claims, namely Claims 55, 56, 58, 60 and 65 are directed to methods wherein the liposomes contain specific drugs, or drugs for specific treatment applications, none of which are disclosed or suggested by the prior art. As such, Claims 55, 56, 58, 60 and 65 can be grouped together.

VIII. Argument

Claims 46-54, 56-57 and 61-64 stand rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Nichols.

In contrast to the present invention, Nichols et al. teaches the uptake of catecholamine by liposomes maintaining a pH gradient. The liposomes of Nichols et al. remain loaded only in the presence of a pre-imposed pH gradient which suggests that upon administration to a host, where the pre-imposed pH gradient no longer exists, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Nichols et al. stated that "[w]hen the gradients were destroyed by ammonium chloride additions, the accumulated catecholamines were released, demonstrating that the uptake was reversible and dependent upon pH gradients." Moreover, Nichols et al. does not disclose administration to a host, and therefore does not teach that the liposome preparation can be administered to animals with no long-term effects. As such, Nichols et al. does not anticipate the present claims.

Claims 46-54, 57 and 61-64 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Deamer et al.

The primary focus of Deamer et al. is to analyze the effects of a pH gradient on flourescent probes. Deamer et al. only uses liposomes to analyze the quenching effect on flourescent probes in the presence a pH gradient. Deamer et al. does not analyze loading liposomes with chemical species to form stable liposome vesicle-entrapped chemical-species, and does not teach that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," or that this stability allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52). Therefore, Deamer et al. does not anticipate the present claims.

Claims 46-54, 59 and 61-64 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Cramer et al. or Kano et al.

Cramer et al. also teaches the physical chemistry involved in using a pH gradient to load certain simple ionizable molecules (fumaric and maleic acid) into a liposome. Kano et al. teaches the use of trisodium 8-hydroxy-1,3,6-pyrene-trisulfonate, pyranine, as a probe for monitoring the pH in the interiors of negatively charged liposomes and at the outer surface of positively charged liposomes. Neither reference teaches or suggests the claimed invention of using a pH gradient to produce a stable liposome vesicle-entrapped chemical species, much less that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," or that this stability allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

The liposomes of Cramer et al. remain loaded only in the presence of a pH gradient which suggests that upon administration to a host, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Cramer et al. states on page 299 (last line) that "following a pH perturbation, an equilibrium condition, corresponding to zero net transport, should be reached where the internal and external H_2A activities are equal." In other words, if liposomes produced in the presence of a pre-imposed pH gradient are placed in an environment where the pre-imposed pH gradient no longer exists, massive amounts of H_2A would leak from the liposomes. Cramer et al. states in the last paragraph on page 300 that non-selective leakage of both the fumaric and maleic acid probably is the result of vesicle rupture in response to osmotic stress. These statements teach away from the present invention where drug entrapped liposomes remain stably loaded after they are injected into a host where the pre-imposed pH gradient used to load the liposomes no longer exists. Upon reading these references, the skilled artisan would not be lead to the present invention. As such, neither Cramer et al. nor Kano et al. anticipates the present claims.

Claims 46-64 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Nichols et al., Deamer et al., Cramer et al. or Kano et al.

Applicant respectfully submits that the subject invention is not taught or suggested by any of the references cited by the Examiner. These references, taken alone or in combination, do not teach or suggest to one skilled in the art that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," or that this stability

allows the animal to suffer "no long-term effects of the administration" (as recited by amended Claims 46 and 52).

It was only with the present invention that liposomes which could be injected into a rat *in vivo* for the delivery of drugs (See Example 3 in the present application) were obtained and recognized as such. Prior to these experiments, one would not have known whether such drug entrapped liposomes would wreak havoc on the biogenic amines that play a vital role in animal physiology. For example, as demonstrated in Deamer et al., catecholamines could be loaded into liposomes with pH gradients. However, until after Applicant's *in vivo* experiments were performed, no one could have predicted that an animal would tolerate the injection of such catecholamine-loaded liposomes, because none of the cited references discloses or suggests liposomes which retain their stability in the absence of a pH gradient across the liposomal membrane.

In the Office Action dated November 16, 2000, the Examiner stated that Applicant's arguments distinguishing the prior art on the basis that it does not show that liposomes containing a therapeutic drug product would retain their contents upon administration to an animal were "not found to be persuasive since the differences argued are not reflected in the claims." (page 3 of the November 16, 2000 Office Action) The independent claims now explicitly recite that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," and that this stability allows the animal to suffer "no long-term effects of the administration", As such, Applicant submits that the claims are patentable over the cited references.

IX. Conclusion

In light of the fact that independent claims explicitly recite that the stability of the liposome is "independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species," and that this stability allows the animal to suffer "no long-term effects of the administration", Applicants submit that the claims patentably define over the cited prior art. As such, withdrawal of the outstanding rejections is warranted, and is thus respectfully requested.

Respectfully submitted,

Burns, Doane, Swecker & Mathis, L.L.P.

Date June 30, 2004

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APPENDIX

The Appealed Claims

46. (Twice Amended) A method for preparing a stable liposome vesicle-entrapped chemical species which comprises the steps of:

(a) forming liposomes comprising a membrane in:

(1) an aqueous medium containing an acid which is substantially impermeable through the vesicle to give an acidic liposome-containing aqueous medium in which the acid is present in the internal and external liposome phases; or

(2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give an basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

(1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or

(2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

(1) a base to provide a pH gradient across the membrane of the liposome and thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or

(2) an acid to provide a pH gradient across the membrane of the liposome and thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical species, said stability being independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.

47. The method according to Claim 46 wherein the chemical species is a drug.

48. The method according to Claim 46 wherein the aqueous medium is a buffer solution.

49. A liposome vesicle-entrapped chemical species prepared by the method of Claim 46.

50. A pharmaceutical preparation for administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49.

51. A pharmaceutical preparation for parenteral administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49, wherein the buffer has an osmolarity within the physiological range of an animal, the vesicles are suspended for administration in a bulk solution, and the bulk solution has a pH which is physiologically benign.

52. (Twice Amended) A method of preparing a stable liposome vesicle-entrapped chemical species, which method comprises:

(a) forming liposomes in:

(1) an aqueous medium containing an acid which is substantially impermeable through the vesicle to give an acidic liposome-containing aqueous medium in which the acid is present in the internal and external liposome phases; or

(2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give an basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

(1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or

(2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

(1) a base in an amount effective to create a pH gradient between the external liposome phase and the internal liposome phase to thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or

(2) an acid in an amount effective to create a pH gradient between the external liposome phase and the internal

liposome phase to thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical species, said stability being independent of maintenance of the pH gradient after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.

53. The method according to Claim 52 wherein the chemical species is a drug.

54. The method of Claim 52, wherein the charged chemical species is a drug having hydrophobic ions.

55. The method of Claim 54, wherein the drug having hydrophobic ions is ellipticinium chloride, an antihelminthic, gentian violet, pyrvinium, pamoate, a cyanine dye, or pamaguine.

56. The method of Claim 53, wherein the drug is a drug for chemotherapy or immunosuppression, a membrane permeable peptide toxin or a hormone.

57. The method of Claim 52, wherein the pH titratable chemical species is a drug having molecules with basic properties.

58. The method of Claim 57, wherein the drug is vincristine, doxorubicin, streptomycin, chloroquine, daunorubicin.

59. The method of Claim 52, wherein the pH titratable chemical species is a drug having molecules with acidic properties.

60. The method of Claim 59, wherein the drug is a derivative of methotrexate, daunomycin, penicillin or a salicylic acid derivative.

61. The method according to Claim 52 wherein the aqueous medium is a buffer solution.

62. A liposome vesicle-entrapped chemical species prepared by the method of Claim 52.

63. A pharmaceutical preparation for administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 62.

64. A pharmaceutical preparation for parenteral administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 62, wherein the buffer has an osmolarity within the physiological range of an animal, the vesicles are suspended for administration in a bulk solution, and the bulk solution has a pH which is physiologically benign.

65. The method of Claim 60, wherein said salicylic acid derivative is p-amino salicylic acid.